

What is claimed is:

1           1. A scalable continuous process for preparing nucleic acid-containing microparticles,  
2 the process comprising:  
3           (a) providing a mixing chamber and a solvent removal device;  
4           (b) continuously supplying a first emulsion to the mixing chamber, wherein the first  
5 emulsion comprises (i) an organic solution comprising a polymeric material and an organic  
6 solvent mixed with (ii) a first aqueous solution comprising a nucleic acid;  
7           (c) continuously supplying a second aqueous solution to the mixing chamber, wherein the  
8 second aqueous solution comprises a surfactant;  
9           (d) continuously emulsifying the first emulsion and the second aqueous solution in the  
10 mixing chamber to form a second emulsion, the second emulsion comprising nucleic acid,  
11 polymeric material, water, and organic solvent;  
12           (e) continuously transferring the second emulsion from the mixing chamber to the solvent  
13 removal device; and  
14           (f) removing the organic solvent from the second emulsion in the solvent removal device  
15 to form an aqueous suspension of nucleic acid-containing microparticles;  
16 wherein at least one of the first emulsion and the second aqueous solution further comprises a  
17 stabilizer.

1           2. The process of claim 1 wherein the first aqueous solution and the second aqueous  
2 solution are of essentially equal osmolarity.

1           3. The process of claim 2, wherein the stabilizer comprises a carbohydrate and a buffer.

1           4. The process of claim 3 wherein the stabilizer comprises sucrose and TRIS-EDTA.

1           5. The process of claim 4 wherein the stabilizer additionally comprises a lipid.

1           6. The process of claim 1 wherein the stabilizer comprises a lipid.

1           7. The process of claim 1, further comprising:

2           (g) providing a diafiltration apparatus;

3           (h) diluting the aqueous suspension with an aqueous wash solution;

4           (i) supplying the diluted aqueous suspension to the diafiltration apparatus; and

5 (j) removing an aqueous waste solution from the diluted aqueous suspension in the  
6 diafiltration apparatus, wherein the aqueous waste solution comprises at least some of the wash  
7 solution of step (h), to form in the diafiltration apparatus a purified aqueous suspension  
8 comprising nucleic acid-containing microparticles.

1 8. The process of claim 7, further comprising:

2 (k) concentrating the purified aqueous suspension in the diafiltration apparatus to form a  
3 concentrate; and

4 (l) transferring the concentrate into one or more vessels.

1 9. The process of claim 8 further comprising:

2 (m) lyophilizing, freeze-drying, or air-drying the concentrate in the one or more vessels,  
3 to form lyophilized, freeze-dried, or air-dried microparticles.

1 10. The process of claim 9 wherein the lyophilized or freeze-dried microparticles have a  
2 residual organic solvent level of less than 200 ppm.

1 11. The process of claim 10 wherein the lyophilized or freeze-dried microparticles have  
2 a residual organic solvent level of less than 50 ppm.

1 12. The process of claim 1, further comprising:

2 (g) contacting the aqueous suspension with a vibrating or non-vibrating fine-mesh screen;

3 (h) filtering the aqueous suspension through the screen to remove at least some of each of  
4 said first and second aqueous solutions and to retain the microparticles on the screen;

5 (i) washing the microparticles with at least one aqueous wash solution to produce washed  
6 microparticles; and

7 (j) drying the washed microparticles to produce dried microparticles.

1 13. The process of claim 12, wherein the drying step comprises lyophilizing, freeze-  
2 drying, or air-drying the washed microparticles.

1 14. The process of claim 12, wherein the first aqueous wash solution is sterile water-for-  
2 injection at a temperature of about 2°C to about 8°C.

1 15. The process of claim 12, further comprising contacting the washed microparticles  
2 with an excipient, prior to the drying step.

1 16. The process of claim 12, further comprising:  
2 (k) transferring the dried microparticles into one or more vessels.

1 17. The process of claim 1, wherein the mixing chamber comprises a homogenizer.

1 18. The process of claim 1, wherein the solvent removal device is a bioreactor.

1 19. The process of claim 1, wherein the second aqueous solution is supplied to the  
2 mixing chamber at a flow rate of between 0.1 and 20 l/min.

1 20. The process of claim 1, wherein the organic solvent is removed from the second  
2 emulsion in the solvent removal device by evaporation.

1 21. The process of claim 1, wherein the organic solvent is removed from the second  
2 emulsion by heating the second emulsion in the solvent removal device to between 30°C and  
3 55°C.

1 22. The process of claim 1, wherein the organic solvent is removed from the second  
2 emulsion in the solvent removal device by an extraction process.

1 23. The process of claim 1, wherein the removal of the organic solvent from the second  
2 emulsion in the solvent removal device is facilitated by diluting the second emulsion in the  
3 solvent removal device.

1 24. The process of claim 1, wherein the organic solvent is removed from the second  
2 emulsion in the solvent removal device by applying a partial vacuum to the solvent removal  
3 device.

1 25. The process of claim 1, wherein the organic solvent comprises dichloromethane.

1 26. The process of claim 9, wherein each of the steps is carried out aseptically.

1 27. The process of claim 7, wherein the diafiltration apparatus comprises a hollow fiber  
2 system.

1 28. The process of claim 7, wherein steps (i) and (j) are carried out at a temperature of  
2 between about 2°C and about 8°C.

1 29. The process of claim 1, wherein at least about 50% of the nucleic acid in the  
2 microparticles is in the form of circular RNA molecules or supercoiled circular DNA molecules.

1 30. The process of claim 7, wherein at least about 50% of the nucleic acid in the  
2 microparticles in the purified aqueous suspension is in the form of circular RNA molecules or  
3 supercoiled circular DNA molecules.

1 31. The process of claim 9, wherein at least about 50% of the nucleic acid in the  
2 lyophilized or freeze-dried microparticles is in the form of supercoiled circular DNA molecules.

1 32. The process of claim 1, wherein the average diameter of microparticles is less than  
2 about 100 microns.

1 33. The process of claim 31, wherein the average diameter is less than about 20 microns.

1 34. The process of claim 32, wherein the average diameter is between about 0.5 and  
2 about 2.5 microns, inclusive.

1 35. The process of claim 1, wherein the polymeric material is a synthetic, biodegradable  
2 polymer.

1 36. The process of claim 35, wherein the polymer is poly-lactic-*co*-glycolic acid  
2 (PLGA).

1 37. The process of claim 36, wherein the ratio of lactic acid to glycolic acid in the PLGA  
2 is between about 1:2 and about 4:1 by weight.

1 38. The process of claim 37, wherein the ratio of lactic acid to glycolic acid in the PLGA  
2 is about 1:1 by weight.

1 39. The process of claim 36, wherein the PLGA has an average molecular weight in the  
2 range of 6,000 to 100,000.

1 40. The process of claim 1, wherein the second aqueous solution further comprises  
2 polyvinyl alcohol (PVA).

1 41. The process of claim 40, wherein the second aqueous solution further comprises a  
2 carbohydrate.

1 42. The process of claim 41, wherein the carbohydrate is sucrose.

1 43. The process of claim 1, wherein the emulsifying step (d) is carried out at between  
2 about 2°C and about 8°C.

1 44. The process of claim 1, wherein the average residence time of the first emulsion and  
2 the second aqueous solution in the mixing chamber is less than about 60 seconds.

1 45. The process of claim 44, wherein the average residence time of the first emulsion and  
2 the second aqueous solution in the mixing chamber is less than about 1 second.

1 46. The process of claim 1, wherein the average residence time of the second emulsion in  
2 the solvent removal device is less than about 3 hours.

1 47. The process of claim 1, further comprising:  
2 (g) providing a diafiltration apparatus;  
3 (h) diluting the aqueous suspension with an aqueous wash solution;  
4 (i) supplying the diluted aqueous suspension to the diafiltration apparatus;  
5 (j) removing an aqueous waste solution from the diluted aqueous suspension in the diafiltration  
6 apparatus, wherein the aqueous waste solution comprises at least some of the wash solution of  
7 step (h), to form in the diafiltration apparatus a purified aqueous suspension comprising nucleic  
8 acid-containing microparticles;  
9 (k) washing the purified aqueous suspension to form a suspension of washed microparticles;  
10 (l) concentrating the suspension of washed microparticles to form a concentrate;  
11 (m) transferring the concentrate into one or more vessels; and

- 12 (n) lyophilizing, freeze-drying, or air-drying the concentrate in the one or more vessels, to form
- 13 lyophilized, freeze-dried, or air-dried powder.